

An Investigation of a Sono-Chemical
Approach in Sterilization Problems

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1. Purpose of Investigation.

The aim of the work being conducted under Grant NoG-684 is to provide some insight into the possible role of air-borne sound in sterilization problems. The combined effects of sound and gaseous agents, such as ethylene oxide, in sterilization problems is also being investigated.

The present report covers work involving the ultrasonic irradiation of spores of B. subtilis var. niger at 50 C and 60 C. In addition, the antibacterial activity of ethylene oxide coupled with ultrasonic irradiation is also reported.

2. Improvements in Methodology.

The test apparatus has now been equipped with a humidity gauge so that continuous monitoring of the humidity levels within the chamber is possible. A second heater has been installed outside the test chamber and temperatures up to 60 C, in the chamber itself, can easily be achieved. A third improvement in the test apparatus was effected by the insertion of a membrane filter in the air line. Sterilization of the recirculated air is now assured. A new ultrasonic transducer (peak = 34.8 kc/sec) has been incorporated into the apparatus.

3. Effect of Ultrasonic Irradiations on the Spores of Bacillus subtilis var. niger at 50 C and 60 C.

The inhibitory effect of sonic and ultrasonic waves on spores of B. subtilis var. niger at 40 C was reported in the second semi-annual report (1 January 1965 - 30 June 1965). In the latter report, data was presented which demonstrated that statistically significant reductions in viable counts of B. subtilis var. niger were possible by exposing spores to sonic and ultrasonic irradiations. In view of the fact that more significant data was obtained with ultrasonic than with sonic waves, additional experiments were designed to include ultrasonic irradiations of B. subtilis var. niger spores at higher temperatures.

An ultrasonic sound source (Friedman Horn) with a peak emission at 34.8 kc/sec was employed. Spores of B. subtilis var. niger were irradiated for periods of from one to eight hours. The spore-containing paper strips were located at 1.0, 3.5, 5.0, and 9.5 inches, respectively, from the sound source. Table 1 presents the data obtained when spores were irradiated at a temperature of 50 C and a relative humidity of 40%.

Table 1. Average counts ($\times 10^6$) of B. subtilis var. niger spores exposed to ultrasonic irradiation (34.8 kc/sec) at 50 C.

Hours	Distance from Transducer, inches			
	9.5	5.0	3.5	1.0
1	1.57	1.51	1.40	1.32
2	1.53	1.37	1.29	1.14
4	1.41	1.48	0.65*	0.99*
8	1.17	1.26	0.69*	0.29*

* denotes statistically significant value at 99% level of certainty.

Statistically significant reductions in the viable count were obtained only after 4 and 8 hours irradiation with the test samples located at 1.0 and 3.5 inches, respectively, from the sound source. Comparing these results with those obtained when spores of B. subtilis var. niger were irradiated with ultrasonic waves at 40 C (Second semi-annual report, 1 January 1965 - 30 June 1965), it is obvious that fewer significant values were obtained at the higher temperature (50 C). Thus, it would appear that the antibacterial activity of ultrasonic irradiation is enhanced by lower temperatures.

In addition to t values, the data obtained at 50 C were expressed in terms of percent reduction of viable count. Figure 1 again demonstrates that reduction in count was directly related to the period of irradiation and the distance of the sample from the transducer. The most significant result occurred when spores were exposed to ultrasonic waves for 8 hours at a distance of 1 inch from the sound source. Increasing the distance of the test sample from the sound source resulted in lower reductions in the viable count.

An investigation of the influence of a temperature of 60 C in ultrasonic irradiation of spores of B. subtilis var. niger was also initiated. Although the results are as yet incomplete, irradiation of B. subtilis var. niger spores at 60 C at a distance of 1 inch from the sound source indicate less significant kill than at either 50 C or 40 C (Table 2).

Table 2. Average counts ($\times 10^6$) and % reduction in viable count of B. subtilis var. niger spores exposed to ultrasonic irradiation (34.8 Kc/sec) at 60 C.

Hours	Distance from Transducer 1 inch	% Reduction in Viable Count
1	1.30	0
2	1.24	2
4	0.96	24
8	0.91*	28

* denotes statistically significant value at 99% level of certainty.

It would appear, based on results obtained previously, that as the distance of the sample is increased from the sound source fewer significant reductions in viable count can be expected.

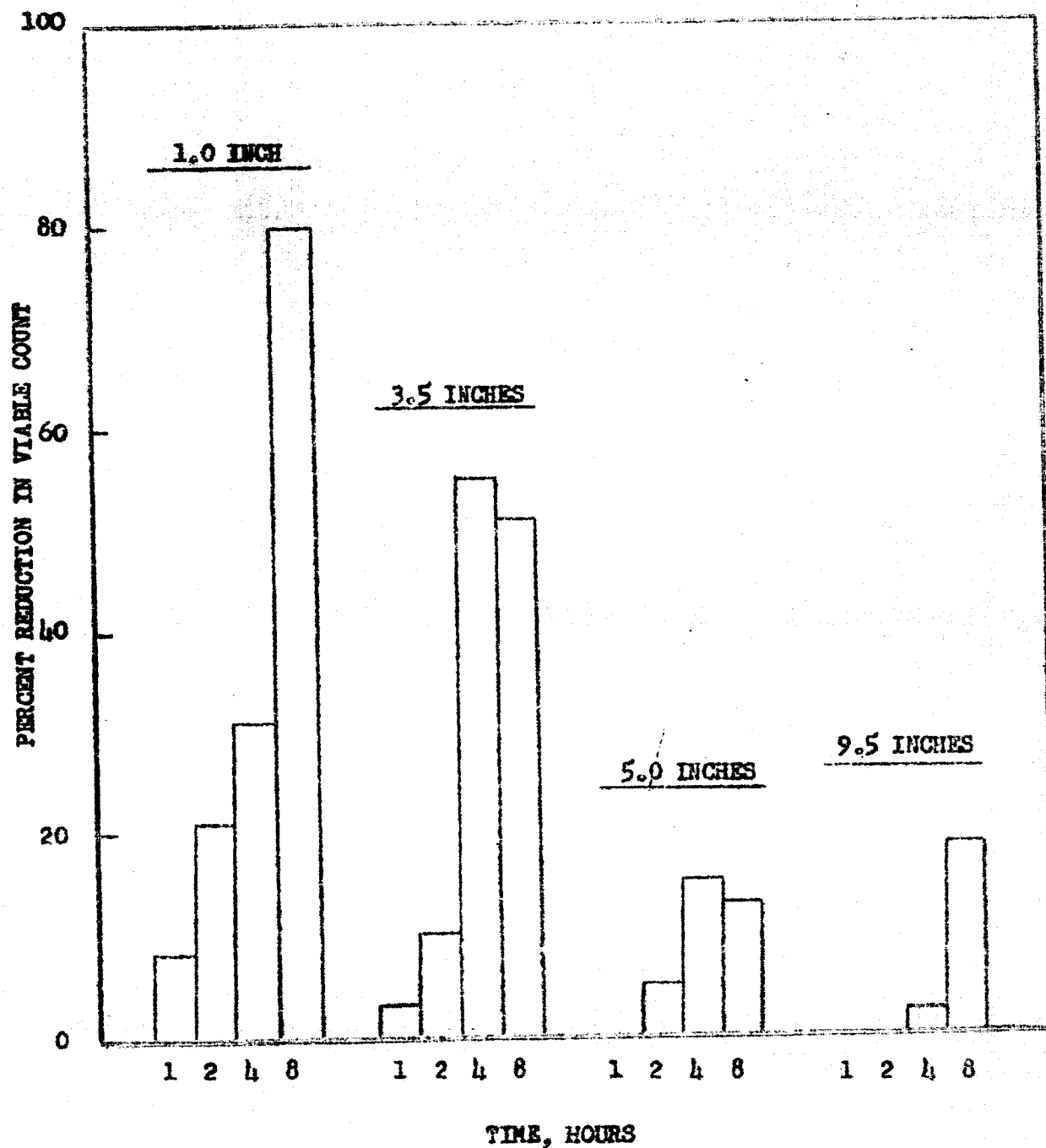


Figure 1. Antibacterial activity of ultrasonic irradiation (34.8 kw/sec) against spores of Bacillus subtilis var. niger at 50 C.

Distance of transducer from test samples is indicated in inches.

4. Effect of Ethylene Oxide and Ultrasonic Irradiation on the Spores of *Bacillus subtilis* var. *niger*.

Efforts to explore the possible use of ultrasonic irradiation in conjunction with ethylene oxide treatment are continuing. In a previous report (second semi-annual report, 1 January 1965 - 30 June 1965), it was shown that statistically significant values were recorded when both agents were used simultaneously. Of importance was the fact that plate counts with ethylene oxide alone, at levels of 125 and 250 mg/liter, were significantly higher than counts obtained with the same concentrations of ethylene oxide but with ultrasonic waves applied. Attempts to duplicate these results have not always proven fruitful. The greatest problem appears to be related to attempts to deliver ETO, in less than 1 ml amounts, with high accuracy into the test chamber. The high volatility of the ethylene oxide presents a serious obstacle to achieving this end. Consequently, a method has been devised for more accurate delivery of the ETO. Rather than adding ETO to the test chamber with a pre-cooled syringe, the ETO is now dispensed in measured aliquots into small test tubes. The latter are equipped with perforated screw-caps over a tightly fitting rubber diaphragm. Prior to making a test run, the tube with the frozen ETO is placed in position to deliver into the test chamber. Volatilization occurs and the ETO is quantitatively transferred to the chamber. Proceeding in this fashion, approximately 1/3 of the trials have shown significant reductions in viable count when ETO is coupled with ultrasound. Table 3 illustrates some representative data.

Table 3. Average counts of spores of B. subtilis var. niger exposed to ethylene oxide (180 mg/liter) alone, and in combination with ultrasound (34.8 kn/sec) at 60 C.

Expt. No.	Without Sound	With Sound
1	845	0
2	170	0
3	175	0
4	710	0
5	255	0

Irradiation distance = 1 inch
Irradiation time = 20 minutes

5. Discussion.

The results obtained thus far indicate that the application of air-borne ultrasonic waves to spores of B. subtilis var. niger will, under certain conditions, cause significant reductions in the population. The extent of reduction is directly related to the period of irradiation, as well as the distance of the test strips from the sound source. In general, the longer the period of irradiation, and the shorter the distance of sample from the sound source, the more significant was the reduction in viable count. Comparing the results reported here with those contained in an earlier report, it appears that the efficacy of ultrasonic irradiation in reducing viable counts is also dependent upon the temperature. Indications are, based on our experiments, that air-borne sound waves are more detrimental to the viability of spores of B. subtilis var. niger at 40 C, rather than at 50 or 60 C. The completion of experiments now in progress will help to more fully clarify the latter observation. The importance of our observations cannot be fully assessed at this time because

of the lack of comparative data in the scientific literature. It is pertinent to cite, however, the work of Frings, Allen, and Rudnick (1948) which described the physical effects of air-borne ultrasonic waves (about 19 kc) of a high intensity (about 1 watt/cm²) produced by a siren. Mice were killed by exposures of approximately one minute, apparently by heat originating from the absorption of sound waves by the fur. In addition to mice, roaches and other insects were also killed by exposure to air-borne sound waves. Our efforts have now demonstrated that air-borne sound may also affect the viability of lower forms of life, and in particular, bacterial spores which are highly resistant to sterilization procedures. Extrapolating from the data cited above, it would appear that appropriate sound sources can be developed which would be able to eliminate or significantly reduce microbial populations which are suspended in air in a variety of environments. Quite often, sterilization of these residual populations is difficult, or practically impossible to accomplish. It is obvious that the potential offered by sound waves towards achieving this end warrants additional study.

The combined effects of ultrasonic waves and ethylene oxide in inhibiting spores of B. subtilis var. niger appears, in some of our experiments, to be superior to the use of either agent alone. The failure of all experiments to show identical trends is attributed to the difficulties attending the transfer of the highly volatile ethylene oxide to our test chamber. Never the less, if strenuous efforts are made to insure the exact transfer of less than 1 ml amounts of ethylene oxide, greater reproducibility of results obtained follows. In view of the difficulty cited, it is attractive to consider additional studies of this type using propylene oxide instead of ethylene oxide. The microbicidal activity of gaseous propylene oxide from a quantitative point of view has been

covered by Bruch and Koesterer (1961). They reported that a 90% kill of spores of B. subtilis var. niger exposed to 1250 mg/liter propylene oxide at 37 C and a relative humidity of 85%. Most attractive is the fact that propylene oxide has a boiling point of 33.9 C which would tend to eliminate many of the problems encountered with the more volatile ethylene oxide.

6. Future Effort.

The investigation of ultrasonic waves on the viability of spores of B. subtilis var. niger will continue. Following the temperature study, it is planned to run experiments which include variations in the frequencies of the sound waves and in the relative humidity. Beyond this, a study of the effect of sound on spores contained on surfaces other than paper is contemplated.

Efforts to stabilize the ethylene oxide experiments will be temporarily halted and propylene oxide will be substituted. It is expected that the use of propylene oxide will eliminate many of the problems coincidental to the ethylene oxide system. Over and above this, the data accumulated with propylene oxide will serve as a basis for comparative experiments involving ethylene oxide.

7. Literature Cited

- a. Bruch, C.W. and M.G. Koesterer. 1961. The microbicidal activity of gaseous propylene oxide and its application to powdered or flaked feed. *J. Food Science* 26: 1-8.
- b. Frings, H., C.H. Allen, and I. Rudnick. 1948. The physical effects of high intensity air-borne ultrasonic waves on animals. *J. Cell. Comp. Physiol.* 31: 339-358.

8. Publication.

As indicated in the last report, a paper summarizing the results of the initial phase of this project is being prepared for publication. A draft of this paper will be submitted as soon as it is available which, most likely, will be within the next 4-6 weeks.